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INEFFECTIVE ERYTHROPOIESIS IN RECIPIENT PRECURSOR CELLS FOLLOWING NON-MYELOABLATIVE STEM CELL TRANSPLANTATION IN PATIENTS WITH SEVERE SICKLE CELL ANEMIA

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After nonmyeloablative (NMA) stem cell transplantation (SCT), we observed greater clinical improvement in sickle cell disease (SCD)-associated symptoms than anticipated by the degree of chimerism established by routine genotyping of marrow or peripheral blood cells in some patients. We hypothesized that RBC chimerism would provide a more accurate assessment of functional donor hematopoiesis. β-globin RNA pyrosequencing can be used to quantify erythroid-specific lineage chimerism following transplant. In this method, direct quantitative sequencing of the sickle mutation in β-globin RNA serves as an informative locus to distinguish between recipient and donor erythropoiesis. This analysis was performed on serial samples collected from 3 patients with severe SCD with HLA-matched related donors who underwent NMA-SCT. β-globin RNA chimerism in peripheral blood (PB) and marrow (BM) was compared to total genomic DNA chimerism and DNA chimerism of phenotypically defined erythroid lineage precursor cells. All patients demonstrated stable mixed donor DNA chimerism in the first 60-100 days, at levels of 25-30%, 40-50% and 56-60% in Pts 1, 2 and 3, respectively, with no difference between PB and BM levels. DNA chimerism of BM erythroid precursors, determined by Y chromosome FISH and ABO staining in Pts 1 and 2, were identical to total BM gDNA chimerism. In contrast, all 3 patients demonstrated a ~2 fold greater level of β-globin RNA chimerism in PB and marrow compared with gDNA chimerism, with 45/55%, 100/100% and 100/100% BM/PB% donor β-globin RNA measured in Pts 1, 2 and 3, respectively. The consistently increased levels of donor derived β-globin RNA detected in marrow samples, compared to the lower levels of erythroid DNA chimerism suggest that recipient erythroid precursor cells with sickle hemoglobin do not effectively mature into peripheral RBC. In patients with mixed hematopoietic chimerism after NMA-SCT, donor erythroid precursor cells have a maturation advantage that allows for greater donor contribution to overall erythropoiesis. This advantage is likely further accentuated by the shortened survival of recipient RBC. Further studies are underway to characterize the mechanisms that lead to ineffective SCD erythropoiesis in these patients.

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BEAM ALLOGENEIC TRANSPLANT IN PATIENTS FAILING AUTOLOGOUS TRANSPLANTATION FOR HODGKIN'S DISEASE OFFERS AN OPPORTUNITY FOR LONG-TERM SURVIVAL

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While the current standard of care for refractory Hodgkin's disease is an autologous stem cell transplant, patients who fail this form of therapy usually receive only palliative therapy. With the possibility of reduced-intensity allografts decreasing toxicity seen previously with ablative transplants and a presumed graft-versus-lymphoma effect, we began a program in 2000 to perform allogeneic transplants with BEAM conditioning in patients with Hodgkin's disease who had failed autologous transplant. We have treated 13 patients to date using graft-versus-host-disease (GVHD) prophylaxis with mini-methotrexate and FK-506 with early weaning from day 60 to day 80. The median age at allograft was 35 (range: 21 to 48). Median time from diagnosis to allograft was 69 months (range: 12 to 172 months), from autograft to relapse was 11 months (range: 0 to 44 months) and from relapse following autograft to allograft was 31 months (range: 2 to 105 months). Median number of relapses from diagnosis was 4 (range: 2 to 6). Of the 13 patients, 11 had chemoresistant disease, 8 had bulky disease (bulk >2 cm in diameter), 3 had extranodal disease and 5 had relapsed disease in a prior radiation port at allograft. Patients received either matched sibling peripheral blood stem cells (5), partially matched sibling bone marrow (1), or matched unrelated bone marrow (7). At a median of 29 months from allograft (range: 2 to 41 months), 6 of 13 patients (46%) are alive and all are currently in complete remission (CR). Of the 7 patients that died, 1 died of sepsis during hospitalization, 2 expired without evidence of disease (GVHD; CHF), and 4 expired due to progressive disease. Nine patients developed chronic GVHD including 5 of 6 in CR (1 too early). Two patients received donor lymphocyte infusions (DLI) following relapse resulting in transient improvement in one and a CR in the other. Given that 46% are alive with no evidence of disease, this compares favorably with accepted indications for allogeneic transplant after failed autograft for Non-Hodgkin's lymphoma. Allogeneic transplant offers a significant opportunity for long-term survival in patients with Hodgkin's disease that have failed autologous transplant, even those with chemoresistant disease and without matched related donors, and should be considered an important option in all such patients.

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[Abstract Withdrawn]

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SINGLE INSTITUTION EXPERIENCE WITH HHV6 INFECTIONS IN THE PEDIATRIC HSCT PATIENTS

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Reactivated Herpes family viral infections continue to cause morbidity and mortality during hematopoietic stem cell transplant (HSCT). Routine monitoring and prophylactic or preemptive therapy for CMV during the peritransplant period are now accepted standards of care in allogeneic HSCT. Other viral infections such as Human Herpesvirus 6 (HHV6) have been increasingly identified as a significant cause of morbidity during HSCT. New technology with quantitative PCR testing permits more rapid diagnosis of HHV6 reactivation. During an eight-month period at our institution five out of nine allogeneic HSCT patients (55%) were diagnosed with HHV6 infection, no autologous patients tested positive. All received acyclovir prophylaxis. Graft source and indication for HSCT were: one matched sibling with MDS; one unrelated cord blood for infant leukemia; and three T-cell depleted matched unrelated transplants for CML. Three of the patients presented with respiratory distress with diffuse alveolar hemorrhage. Bronchial alveolar lavage (BAL) was positive for HHV6 by PCR on all three patients. The other two had central nervous system (CNS) manifestation; one had a seizure and the other had progressive mental status changes. Spinal fluids from both patients were positive for HHV6 by PCR. Four of the patients were treated with Ganciclovir; the fifth patient received Foscarnet since this patient was already on Ganciclovir for CMV infection. Two of the patients responded initially to the Ganciclovir with a decrease in HHV6 quantitative PCR level, but the PCR increased and symptoms worsened requiring a switch to Foscarnet. The other three continued to have progressive deterioration from the HHV6. HHV6 was the cause of death in all five patients. These case reports demonstrate the emergence of HHV6 as a significant viral pathogen in allogeneic HSCT patients. Vigilant clinical observation with routine monitoring of HHV6 by a sensitive and reliable test and prompt treatment with appropriate antiviral agents may allow improved outcome for this deadly viral infection.

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T CELL (CD3+) CHIMERISM AFTER NON-MYELOABLATIVE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (NMHSCT)

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NMHSCT may potentially achieve a graft-vs-malignancy effect mediated by donor-derived T cells. From 1/10/00 through 9/9/03 we treated 50 pts with NMHSCT using a preparative regimen of fludarabine 30 mg/m^2/d IV on days -5, -4, -3, and total body irradiation 200 cGy on day -1. Diagnoses included 12 NHL, 9 AML, 7 CML, 6 myeloma, 4 MDS, 4 MFB, 4 renal cell carcinoma, 2 CLL and 2 Hodgkin's lymphoma. The median CD34+ and CD3+ cell doses infused were 6.64 x 10^6/kg and 3.74 x 10^8/kg, respectively. 14 pts had matched unrelated donors (MUD). T cell (CD3+) chimerism was monitored by short tandem repeat analysis. Mixed chimerism (MC) consisted of ≥1% to ≤99% host DNA. Thirty (60%) pts achieved T cell complete donor chimerism (CDC) at a median of 63 days (range 14-400) with 7 (50%) MUD pts and 23 (64%) related donor pts achieving CDC. 32 (64%) pts developed acute GVHD at a median of 34 days (range 8-97) with 12 grade 1, 7 grade 2, 8 grade 3 and 5 grade 4. At the time acute GVHD developed the median % of donor T cell DNA was 90% (range 37-100) and only 11 (34%) of these pts had achieved CDC. 19 (38%) pts developed chronic GVHD at a median of 196 days (range 104-531) and at the time of its detection 15 (79%) pts had achieved CDC. GVHD developed in 9 (64%) MUD pts and in 29 (81%) related donor pts. There were 19 (38%) pts with either disease relapse/progression at a median of 117 days (range 17-639) which occurred in 8 pts with CDC, in 8 pts with MC and in 3 pts with graft failure. 13 (26%) pts achieved CR with 9 having stable CDC, 3 with stable MC without ever achieving CDC and 1 pt had a graft failure. 6 pts achieved a PR with 3 having stable CDC, 2 with MC without ever achieving CDC, and 1 pt not evaluable for T cell chimerism. At 10 months median follow-up (range 0.7-42 mos) 25 (50%) pts have died. 11 (44%) of these pts had stable CDC, 7 never achieved CDC with 3 of them proceeding to a full ablative HSCT, 5 achieved CDC which reverted to MC and then back to stable CDC, 1 achieved CDC but due to conversion to MC underwent a full ablative HSCT, and 1 pt was not evaluable for T cell chimerism. We conclude that post-transplant monitoring of T cell (CD3+) chimerism is important to allow for immune manipulation to maintain a state of donor-host tolerance in order to prevent graft rejection. Acute GVHD commonly occurs after NMHSCT without achieving CDC, whereas pts who develop chronic GVHD as well as those who attain disease responses are more likely to have CDC.

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ONE HUNDRED CHILDREN UNDERGOING ALLOGENEIC BONE MARROW TRANSPLANTATION WITH CYCLOPSPORIN (NEORAL) BY MOUTH

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Bone marrow transplantation is performed as an elective therapy in children affected by onco-haeamtological and genetic diseases. Since 1996 we have grafted 100 children affected by onco-haeamtological diseases from haploidentical (14), matched unrelated (60), identical (26) by using cyclosporin (Neoral) by mouth. In all cases cyclosporin have been administred all through the transplant, without using the drug IV. All patients receive preaparative chemiotherapy regimens before transplant, among our patients , 25 affected by acute leukemias received total body irradiation. Mean age at transplant was 3,5 years (age range 1 month-12 years). In all cases cyclosporin levels were within the normal range. Incidence of acute GVHD grade III/IV was 12%, chronic GVHD 4 %.Transplant related mortality was 18%. None of the children presented side effects related by cyclosporin administration. Our experience demonstraed that in pediatrics there is no need of intravenous administrationof cyclopsorin.

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HAPLOIDENTICAL TRANSPLANTS FROM POSITIVELY SELECTED BONE MARROW HAEMATOPOIETIC STEM CELLS: REPORT OF 20 CASES

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Genetic diseases affecting haematopoietic and immune systems can be cured by bone marrow transplantation (BMT). Haploidentical BMT is the treatment of choice in many cases since is uneven to have an healthy matched family donor in family trees affecte by a genetic disease. Moreover the search of matched unrelated donor can take long (3-6 months)and succeed in 70% of the cases. Since 1983 haploidentical traplants have been performed by T cell depletion. In our experience we have performed 40 haploidentical transplants by Campath 1M bone marrow T cell depletion in children affected by genetic diseases from 1991 to 1996. The overall engraftment rate was 60%, but the event free survival did not exceeded 40%. Camtpath 1M recognized CD52 positive cells, that meant T cells, B cells, monocytes, plasmacells and approximately 50% of CD34+ cells. Reasons of relative unsuccess were maybe related to a profound depltion of accesory cells within the bone marrow suspension. Since 1996 we utilized Clinimacs clinical device to perform positive selection of haetopoietic stem cells. Since we are treating genetic diseases the children affected have a mean age of 2,5 years, therefore the weight at transplant do not exceed 10 kg. We have performed 20 haploidentical transplants positively selecting haematopoietic stem cells from bone marrow. The children were affected by severe combined immunodeficinecies(SCID) and other primary immunodeficiencies. The mean yield of stem cells was 75% of the initial stem cell count, while the purity of the stem cells was 91. 8%. Engraftment rate was 90% with a mean time for complete immune reconstitution that did not exceed 3 months.

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ALLOGENEIC STEM CELL TRANSPLANTATION FOR THE TREATMENT OF 12 PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA IN BRAZIL

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Background: Paroxysmal Nocturnal Hemoglobinuria (PNH) is a clonal disorder caused by acquired mutations of the PIG-A gene. Stem Cell Transplantation (SCT) with an HLA identical sibling is potentially curative for young patients with severe pancytopenia, massive hemolysis or serious thrombotic complications. Material and Methods: We performed a retrospective analysis of 12 pts who underwent a non T cell depleted SCT from HLA identical donors at our institution between 03/1988 and 04/2003. Indication for SCT: severe pancytopenia: 9 pts, thrombosis: 2 pts and hemolysis: 1 pt. The conditioning regimen consisted of BU12mg/kg + CY 120mg/kg: 8 pts (1 pt received BU16mg/kg), BU 8mg/kg + Fludarabine 125mg/m2: 1 pt and CY 200mg/kg: 1 pt. Bone marrow was the stem cell source for 11 pts while 1 pt received peripheral blood. All but one pt received methotrexate + cyclosporine as graft versus host disease (GVHD) prophylaxis. All pts received prophylactic antibiotics with fluconazol, sulfamethoxazole-trimethoprim and acyclovir according to common practice. Results: Pts characteristics: Median age at transplantation: 27, 5 years (range: 14-42 yr); Gender: 4F/8M. Time from diagnosis to SCT was a median of 16 months (range: 2-133m). A median of 22, 5 UI of blood transfusions were given before transplant (range: 8-200 UI). Total nucleated cell infused: 1, 72-12, 3 x 10^8/kg (Median: 2, 74). All pts surviving more than 28 days engrafted at a median of 18 days (range: 15-25 days). Mucosytis grade III-IV occurred in 6 pts. Two patients developed grade III-IV acute GVHD. Two pts had extensive chronic GVHD : One progressed after A-GVHD and the other one had a *de novo* extensive C-GVHD. No pt developed veno-occlusive disease. Eight pts are alive and well with a median follow up of 3038 days (range: 176-5662). Four pts (Median age: 36,5yr) died on day + 10, + 11, + 71 and + 330 after SCT. Causes of death included infection (2) and GVHD (2).Estimated 9 year overall survival is 66% (95% CI). Conclusion: We conclude that pts with PNH and life threatening complications can achieve long term survival after this conditioning regimen. These pts can be offered an allogeneic STC if they have an HLA identical sibling and develop severe pancytopenia, hemolysis or recurrent thrombosis.